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Critical Quality Attributes and Manufacturing Process Control Strategies of Antibody–Drug Conjugates: AQbD/Risk Assessment Perspective

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Abstract. Antibody–drug conjugates (ADCs) have emerged as an important class of targeted cancer therapeutics by combining the specificity of monoclonal antibodies with the potency of cytotoxic payloads. Their complex molecular structure and multi-step manufacturing process, however, there are significant challenges for product consistency, characterization and quality control. The key critical quality attributes (CQAs) of ADCs are discussed in this essay, such as drug-to-antibody ratio, conjugation heterogeneity, aggregation, fragmentation, charge variants, linker stability, and residual free payload, as well as biological activity. It also examines the impact of these attributes upon the quality of the antibody, the properties of the linker–payload, the conditions of the conjugation procedure, and others. Special emphasis is placed on the use of Quality by Design and risk assessment in the connection of the QTPP with the CQAs, critical material attributes and critical process parameters. The analysis suggests that only an integrated, science based control strategy over the entire product lifecycle can ensure the quality of ADC, not just end product testing. However, the complexity and products' specificity of the ADCs might reduce the ability to apply a universal control framework directly. Therefore, process understanding and the development of more sensitive analytical technologies, as well as product-specific risk assessment approaches should be emphasized in the future to facilitate safer, more consistent and more robust ADC manufacture.

Keywords: Antibody–drug conjugates, Critical quality attributes, Quality by Design, Quality risk assessment, Manufacturing process control

1. Introduction

Antibody-drug conjugates (ADCs), have become a significant class of targeted cancer therapeutics. An ADC is generally made up of 3 parts: a monoclonal antibody (mAb), a chemical linker, and a cytotoxic payload. The antibody is used for recognizing tumor-associated antigens specifically. The linker connects the antibody to the payload and controls drug release [1]. In principle, this design enables the more targeted delivery of cytotoxic drugs to tumor cells without exposing normal tissue. Unlike conventional cancer-treating therapies, which often affect both tumor cells and normal

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tissues, the cytotoxic drug is targeted more specifically to tumor cells in the case of ADCs. This may help minimize systemic toxicity while maintaining anti-tumor efficacy.

Nevertheless, despite the clear therapeutic benefits, ADCs' complex structure makes manufacturing an control of ADCs challenging. MAbs are already complex biological products, and ADCs introduce an extra chemical conjugation step, attaching the payload to the antibody. This necessary step can ensure a heterogeneous mixture rather than a single uniform product [2]. There are various types of ADC molecules, and the number of payload molecules attached to each molecule may vary to each molecule. As a result, critical quality attributes need to be monitored and controlled, such as drug-to-antibody ratio (DAR), DAR distribution, conjugation site heterogeneity, aggregation, fragmentation, charge variants, linker stability, and residual free payload [2].

These quality attributes are manufacturing concerns as well as can have a direct impact on the clinical efficacy of ADCs. So, critical quality attributes (CQAs) identification and control play an important role in ensuring the safety, effectiveness and batch consistency of ADC products [2]. The QC of ADC products must not just be based on the end product, as the quality of the end product is dependent on antibody, linker-payload, conjugation, purification, and formulation steps of the manufacturing process. These challenges can be addressed with a Quality by Design (QbD) approach that incorporates quality control into the manufacturing process, rather than at the end of the product. Here, the Quality Target Product Profile (QTPP), CQAs, critical material attributes (CMAs), and critical process parameters (CPPs) are identified. Risk assessment tools may also be useful to further identify which quality risks will be given greater priority in controlling them. From this framework, the following CQAs of ADCs will be discussed and the use of manufacturing control strategies to assure product safety, efficacy and batch to batch consistency will be analyzed.

2. ADC structure and key quality challenges

2.1. Main structural components of ADCs

ADCs are a combination of the potency of anti-tumor toxic drugs (which is often called payloads) with the specificity of mAbs to the tumor site. As such, chemotherapy and immunotherapy is used together. The three components of ADCs are the monoclonal antibody, the linker and the cytotoxic payload [1]. All components contribute to the targeting capability, stability and therapeutic activity of ADCs in their own unique yet interdependent ways. The targeting module of an ADC is the antibody portion, which specifically binds to the binding sites on the cancer cells. The binding sites may be tumor-associated antigens present on the surface of the cancer cells. This selective binding enables the ADC to concentrate at the tumor location and enables further ADC-antigen uptake. Hence, some changes, which include aggregation, glycosylation, integrity, antigen-binding activity and charge variants, may affect the final ADC product. The influence may contain specificity, stability, immunogenicity, and biological activity of the final ADC product. The linker not only functions as a chemical bridge between the payload and the antibody, but also plays a critical role in controlling the release of the payload [3]. An ideal linker should maintain sufficient stability during circulation but enable efficient payload release after reaching the target site. If the linker is insufficiently stable, premature payload release may occur before the ADC reaches tumor cells, resulting in reduced targeting efficiency and increased systemic toxicity. In contrast, excessive linker stability may prevent efficient payload release inside tumor cells, thereby reducing the cytotoxic activity of the ADC. Since then, linker properties are closely associated with CQAs such as linker stability, residual free payload, product-related impurities, and biological activity.

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Generally, payloads are highly potent cytotoxic agents because only a limited proportion of administered ADCs reaches tumor tissues. As for the physicochemical properties of the payload, especially hydrophobicity and chemical stability, may influence DAR, residual free payload, aggregation tendency, and biological potency.

2.2. Functional interdependence and quality challenges

As for ADCs, their therapeutic performance requires high target binding specificity, a potent payload, a stable linker, efficient internalization and low immunogenicity. The therapeutic performance of ADCs therefore depends on the precise optimization of these three components [4]. An effective ADC requires a balance between antibody-mediated targeting, linker-controlled drug release, and payload-induced cytotoxicity. Any imbalance among these components may compromise the therapeutic index of ADCs by reducing antitumor activity or increasing systemic toxicity. Therefore, although the combination of these components provides ADCs with their unique therapeutic advantages, it also introduces additional challenges during ADC development and manufacturing. Owing to the complex formation and structure of ADCs, variations introduced during development and manufacturing may significantly affect their therapeutic performance. Since ADCs consist of both biological and chemical components, changes in antibody properties, conjugation-related characteristics, or payload loading may influence product stability, safety, efficacy and pharmacokinetic behavior [5]. For instance, uncontrolled variations in drug loading or molecular heterogeneity may result in ADC species with different biological activities and toxicity profiles. In particular, there are some differences in the number and location of conjugated payload molecules. These kinds of differences would lead to ADC species with different DAR values, hydrophobicity, stability, pharmacokinetic behavior, and biological activity. Therefore, maintaining consistent product quality is essential for ensuring the safety and effectiveness of ADC therapies. Identification and control of CQAs are consequently important aspects of ADC development and manufacturing. These disconnected quality variations form the basis for identifying the major CQAs discussed in the following section.

3. CQAs of ADCs

Among the CQAs of ADCs, drug-to-antibody ratio (DAR), which is defined as the average number of payload molecules attached to each antibody molecule. The reason why DAR takes such a significant role is that it determines the amount of cytotoxic payload delivered to target cells and can directly influence therapeutic efficacy and safety [6]. Nevertheless, DAR needs to be carefully optimized rather than simply maximized. A relatively low DAR may reduce the amount of payload that is delivered to tumor cells and weaken antitumor activity. In contrast, an excessively high DAR can increase ADC hydrophobicity, which may promote aggregation, decrease molecular stability, accelerate clearance, and increase toxicity [4]. Therefore, both the average DAR and DAR distribution should be controlled to maintain a proper balance between efficacy and toxicity. In addition to the average DAR, conjugation heterogeneity should also be considered because ADC molecules may differ in both the number and location of attached payloads. These variations can generate ADC species with different hydrophobicity, stability, clearance rates, biological activity, and toxicity profiles [2].

In addition to DAR-related heterogeneity, aggregation, fragmentation, and charge variants are also important CQAs for ADCs. Aggregation is especially important since many of the chemistry payloads are hydrophobic and they can cause aggregation of the ADC during its manufacture or

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storage when added to the antibody. Other processing or storage conditions such as changes in temperature, agitation, pH or freeze-thaw stress can also promote aggregation. Aggregated species can affect product stability and contribute to immunogenicity and may also impact on pharmacokinetics and drug exposure [6]. Antibody fragments can be produced by proteolysis, disulfide bond disruption, chemical degradation, mechanical and thermal stress during production and storage. Variants may be due to chemical or post-translational events like: These mutations may alter the physicochemical characteristics of the ADC and potentially affect binding, stability or clearance [2, 6]. Therefore, aggregation, fragmentation, and charge variants should be carefully monitored to maintain ADC stability, biological function, and batch-to-batch consistency.

There are many contributors that lead to ADC toxicity, and premature release of payload into bloodstream circulation takes an important role in these. Recent clinical evidence has shown that higher systemic free-payload concentrations were related to higher DAR values. Some experimental results found out that ADCs with cleavable linkers lead to premature payload release, resulting in increased free payload concentrations and related toxicities [7]. In that case, the linkers play a pivotal role in ensuring stability during ADC circulation in the bloodstream. At the same time, it should also allow the payload to be released after the ADC enters the target cell [8]. As for biological activity, it's another significant CQA because it reflects the overall functional performance of an ADC. Several interconnected processes may influence effective biological activity, including internalization, subsequent cytotoxic effects, intracellular linker stability and antigen binding. It is possible that changes in antibody structure, payload loading and linker stability may impair these processes and affect therapeutic efficacy. During the process of quality control, biological activity should be evaluated routinely, so that the final ADC product retaining its intended therapeutic function can be ensured.

4. Manufacturing and analytical control strategies

The above identification of CQAs is mentioned; however, it's just the first step in ADC quality control. Appropriate manufacturing control strategies should also be implemented throughout the production process, making sure that these quality attributes remain within the desired range. In a QbD way, not only relying on end-product testing, but product quality should also be built into the manufacturing process. Accordingly, manufacturing control should begin with critical starting materials and upstream process design, because variations introduced during these stages may directly affect multiple CQAs and ultimately determine the consistency of ADC products [9].

The first stage of upstream control focuses on the quality of the antibody and linker-payload starting materials. Specifications should be established for antibody integrity and aggregation, as well as for the purity and impurity profile of the linker-payload components [9]. Particular attention should be given to conjugatable, chiral, and genotoxic impurities, because these may interfere with the conjugation process and affect product consistency. Among the ADC manufacturing steps, the conjugation process is one of the most critical steps since it determines the drug-loading profile and product heterogeneity. Conventional lysine conjugation often generates a broad distribution of conjugated species, whereas cysteine-based conjugation generally provides better control over DAR and product homogeneity [10]. Site-specific conjugation technologies have been developed to further improve product consistency. Regardless of the conjugation strategy used, reaction conditions should be optimized to ensure reproducible product quality.

Following conjugation, downstream process control is required to remove process-related impurities and preserve stability of ADCs. Aiming to eliminate linker-payload species, residual free payload, unconjugated antibodies, and other process-related impurities, purification may affect

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product quality and increase off-target toxicity [9]. After purification, formulation and storage conditions should be optimized to minimize degradation pathways such as aggregation, linker cleavage and fragmentation. During the process of storage and transportation, those external factors such as oxidative stress, mechanical stress, light exposure, and temperature may even further compromise ADC stability. Hence, appropriate formulation design, excipient selection, packaging, and storage conditions are essential to maintain product quality throughout the product lifecycle [11].

It is essential of analytical characterization to verify whether the predefined CQAs have been maintained throughout manufacturing. No single analytical technique is sufficient to fully characterize their structural complexity because of the biological and small-molecule components [12]. Instead, multiple orthogonal analytical methods should be used to evaluate different quality attributes. For instance, hydrophobic interaction chromatography (HIC) and LC-MS are commonly applied to determine DAR and drug-load distribution, whereas size-exclusion chromatography (SEC) and CE-SDC are used to detect aggregates and fragments. Charge variants can be characterized using ion-exchange chromatography (IEX) or imaged capillary isoelectric focusing (icIEF), while biological activity should be confirmed by antigen-binding and cell-based potency assays. Together, these complementary analytical methods provide comprehensive evidence f ADC identity, purity, structural integrity and biological function.

5. QbD-based risk assessment and control strategy

Although manufacturing and analytical controls can reduce process variability, they should not be considered as independent quality activities. Instead, these control measures need to be integrated into a systematic development framework, which is provided by QbD. For pharmaceutical products, QbD emphasizes that product quality should be built into the manufacturing process through scientific understanding and risk-based decision-making instead of depending solely on end-product testing [13]. Within this framework, pharmaceutical development begins with establishing the Quality Target Product Profile (QTPP), which defines the desired quality characteristics of the final product. With the basement of the QTPP, to identify product safety and efficacy, CQAs are essential. Material attrivutes and process parameters are assessed based on how strongly they may affect the identifies CQAs. Those with a significant impact are classified as CMAs or CPPs to be determined and incorporated into and appropriate control strategy. This systematic relationship provides the scientific basis for manufacturing operations, including process design, optimization, and quality control [13].

The QbD frameworks particularly beneficial for ADCs as many CQAs such as DAR, aggregation, linker stability, free payload and biological activity can be impacted concurrently by material attrivutes manufacturing conditions. Hence, CMAs and CPPs should be ranked according to their potential impact on CQAs, so that resources can be focused on the highest risk quality issues, and provide the basis for subsequent quality risk assessment and control strategy development [9, 14]. Risk assessment is required to identify stricter control, because nor all CQAs contribute to product quality. According to ICH Q9, quality risk management should be based on scientific knowledge and linked to patient protection. The level of control should also be proportional to the significance of the identified risk [14].

Among the CQAs of ADCs, DAR, aggregation, linker stability, and residual free payload usually require the closest attention, as changes in these attributes can affect both efficacy and systemic toxicity. Control efforts should therefore be concentrated on key manufacturing stages such as

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conjugation, purification and formulation. Prioritizing these steps helps maintain product consistency and ensures that resources are directed toward the most significant quality risks [9, 14].

The control strategy should be effective throughout the whole product lifecycle, instead of concentrating only on a special manufacturing step. According to ICH Q10, the quality of the product should be supported by ongoing process monitoring, knowledge management, change management and continual improvement to be in a state of control [15]. This means unifying material control, manufacturing process control, analytical characterization, and quality risk management into a single quality system for ADCs. The information from manufacture and product testing should be reviewed continuously to optimise performance of the process and to update control strategies where appropriate. This integrated lifecycle approach helps to ensure consistent product quality, patient safety and long-term manufacturing reliability.

6. Conclusion

This review discusses the major CQAs of ADCs such as drug-to-antibody ratio, conjugation heterogeneity, aggregation, etc. These attributes are directly related to product safety, efficacy, stability, pharmacokinetic properties and batch-to-batch consistency. The analysis reveals that the quality of ADC is affected by several steps of development and manufacturing such as antibody production, linker–payload preparation, conjugation, purification, formulation, storage and analytical testing.

The testing of final product should not be the exclusive measure of quality control in the context of effective ADC. Rather, a framework is required that connects the Quality Target Product Profile to CQAs, critical material attributes and CPPs; this is known as a QbD approach. Risk assessment can also be used to prioritize the most significant quality risks and manufacturing steps and Lifecycle monitoring can help with continuous process control and improvement. Because of the diversity of the ADC structures, antibody chemistry, payload characteristics and technologies used for ADCs, a universal control strategy for all products may not be available. More research into the processes, more sensitive and orthogonal analytical methods, and more product-specific risk assessment strategies should thus be prioritized for the future. Overall a combination of science and risk based approach is critical to further strengthen the strength of manufacturing and to provide consistent manufacturing of safe and effective ADC products.

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